Alkaline Phosphatase Assay Kit

Catalog No.: abx096002

Size: 100 Assays

Storage: Store all kit components in the dark at 4°C.

Application: For quantitative detection of alkaline phosphatase activity in serum, plasma, tissue homogenates, cell lysates, cell culture supernatants, urine, and other biological fluids.

Detection Range: 0.05 mmol/L – 5 mmol/L

Introduction: Alkaline phosphatases (ALPs) are a group of enzymes that catalyze the hydrolysis of phosphate esters at alkaline pH. In mammals, ALP is primarily found in the liver, kidney, and bone. High serum ALP concentrations are associated with primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), sarcoidosis, malignant biliary obstruction, and hepatic lymphoma.

ALP hydrolyzes disodium phenyl phosphate to a phenol and phosphate product. The phenol product forms a colored complex with 4-aminoantipyrine in the presence of potassium ferricyanide at pH 10. The concentration of the reaction product is directly proportional to the enzyme activity, which can be measured by measuring the absorbance at 510 nm.

Kit components

1. 96 well microplate
2. Assay Buffer: 4 × 30 ml
3. Reaction Buffer: 4 ml
4. Standard: 1 vial
5. Positive Control: 0.1 µl
6. Dye Reagent 1: 1 vial
7. Dye Reagent 2: 1 vial
8. Substrate: 1 vial
9. Plate Sealer: 3

Materials Required But Not Provided

1. Microplate reader (510 nm)
2. High-precision pipette and sterile pipette tips
3. Distilled water
4. Mortar
5. Centrifuge and centrifuge tubes
6. Timer
7. Ice
8. Sonicator

Protocol

A. Preparation of Sample and Reagents

1. Reagents

- **Substrate Solution**
  Add 4 ml of distilled water into the Substrate vial and mix thoroughly to prepare the Substrate Solution. Ensure that the Substrate has completely dissolved prior to use.

- **Dye Reagent 1 Solution**
  Add 10 ml of distilled water into the Dye Reagent 1 vial and mix thoroughly to prepare the Dye Reagent 1 Solution. Ensure that the Dye Reagent 1 has completely dissolved prior to use.

- **Dye Reagent 2 Solution**
  Add 2 ml of distilled water into the Dye Reagent 2 vial and mix thoroughly to prepare the Dye Reagent 2 Solution. Ensure that the Dye Reagent 2 has completely dissolved prior to use.

- **Standard Solution**
  Add 1 ml of distilled water to the Standard vial and mix thoroughly. Add 50 µl of this solution to 950 µl of distilled water to prepare a 1 ml Standard Solution with concentration 5 mmol/L.

- **Positive Control Solution**
  Add 1 ml of Assay Buffer to the Positive Control vial and mix thoroughly to prepare the Positive Control Solution. Ensure that the Positive Control has completely dissolved prior to use.

2. Sample

- **Cell and Bacterial samples**
  Count the number of cells in the culture. Collect the cells into a centrifuge tube, and centrifuge to precipitate the cells. Discard the supernatant and add 1 ml of Assay Buffer for every 5,000,000 cells. Sonicate at 20% power in 3 second bursts with a 10 second rest interval, for 30 bursts in total. Centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

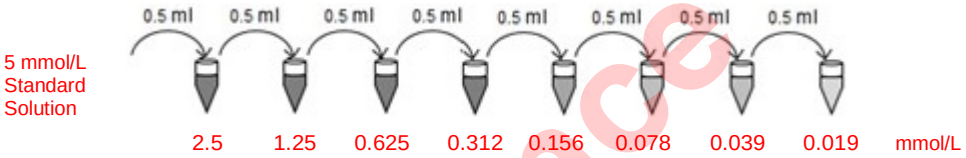
- **Tissue samples**
  Homogenize 0.1 g of sample in 1 ml of Assay Buffer on ice, then centrifuge at 8000 × g at 4°C for 10 minutes. Transfer the supernatant to a new tube, keep on ice and analyze immediately.

- **Serum and Plasma samples**
  Serum and plasma samples can be used directly or diluted with Assay Buffer if required.

B. Assay Procedure

Bring all reagents to room temperature prior to use.

1. Label 8 tubes with 2.5 mmol/L, 1.25 mmol/L, 0.625 mmol/L, 0.312 mmol/L, 0.156 mmol/L, 0.078 mmol/L, 0.039 mmol/L and 0.019 mmol/L. Aliquot 0.5 ml of distilled water into each tube. Add 0.5 ml of 4 mmol/L Standard Solution to the 1st tube (2 mmol/L) and mix thoroughly. Transfer 0.5 ml from the 1st tube to the 2nd tube and mix thoroughly, and so on.



2. Set the sample, standard, blank, and positive control wells and record their positions. We recommend setting up each standard and sample in duplicate.
3. Add 10 µl of sample to sample wells.
4. Add 10 µl of prepared standard solutions to the standard wells.
5. Add 10 µl of distilled water to the blank wells.
6. Add 10 µl of Positive Control solution to the positive control wells.
7. Add 40 µl of Reaction Buffer to all wells.
8. Add 40 µl of Substrate Solution to all wells.
9. Tap the plate gently to mix. Incubate at 37°C for 15 minutes.
10. Add 90 µl of Dye Reagent 1 Solution to all wells.
11. Add 20 µl of Dye Reagent 2 Solution to all wells.
12. Tap the plate gently to mix. Allow to stand for 10 minutes.
13. Read and record absorbance at 510 nm.

C. Calculations

One unit of ALP activity is defined as the amount of enzyme required to produce 1 nmol of phenol per minute.

Alkaline phosphatase activity per mg of protein:

$$\mathbf{ALP\ (U/mg)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{C_{Protein}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{333.33}}{\mathbf{C_{Protein}}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Alkaline phosphatase activity per g of sample:

$$\mathbf{ALP\ (U/g)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}} \times \mathbf{V_{Assay}}}{\mathbf{V_{Sample}} \times \mathbf{W} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \frac{\mathbf{333.33}}{\mathbf{W}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

Alkaline phosphatase activity per ml of serum or plasma:

$$\mathbf{ALP\ (U/ml)} = \frac{\mathbf{C_{Standard}} \times \mathbf{V_{Standard}}}{\mathbf{V_{Sample}} \times \mathbf{T}} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}} = \mathbf{333.33} \times \frac{\mathbf{OD_{Sample}} - \mathbf{OD_{Blank}}}{\mathbf{OD_{Standard}} - \mathbf{OD_{Blank}}}$$

where:

$\mathbf{C_{Protein}}$	Concentration of protein (in mg/ml)
$\mathbf{C_{Standard}}$	Concentration of highest standard (5 mmol/L = 5000 nmol/ml)
$\mathbf{T}$	Reaction time (15 minutes)
$\mathbf{W}$	Weight of the sample (in g)
$\mathbf{V_{Assay}}$	Volume of assay buffer (1 ml)
$\mathbf{V_{Sample}}$	Volume of sample (0.01 ml)
$\mathbf{V_{Standard}}$	Volume of standard (0.01 ml)